Biomass Production of Marine Microalgae as Feedstock for Bioenergy in Synthetic Urea Wastewater

Gusti Diansyah¹, Riris Aryawati², M. Akbar Rahman³, Lisya Meidina⁴, Karin Miranda Pasya⁵, Tengku Zia Uldry⁶

{gusti.diansyah@unsri.ac.id¹, riris.aryawati@unsri.ac.id², 20012622327011@student.unsri.ac.id³, 08051282025061@student.unsri.ac.id⁴, 08051282025043@student.unsri.ac.id⁵, zia_uul@unsri.ac.id⁶}

Department of Marine Science, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Ogan Ilir 30662, South Sumatra, Indonesia¹,²,⁴,⁵,⁶

Doctoral Program of Environmental Science, Graduate Program, Universitas Sriwijaya, Palembang 30139, South Sumatra, Indonesia³

Environmental Management Study Program, Graduate Program, Universitas Sriwijaya, Palembang 30139, South Sumatra, Indonesia³

Correspondent author: gusti.diansyah@unsri.ac.id

Abstract. Growth rate of marine microalgae (Nannochloropsis oculata, Tetraselmis chuii, and Porphyridium sp.) in urea wastewater based medium is investigated. Microalgae were cultivated in sterile seawater under controlled conditions with varying amounts of urea: 0 mg/L (as a control variable), 25 mg/L, 50 mg/L, 75 mg/L, 100 mg/L, and 150 mg/L. During the experiment, environmental parameters, including pH, temperature, dissolved oxygen (DO), and salinity, were monitored. The study showed that the microalgae cell growth rate of Nannochloropsis oculata was 0.44 x 10⁴ cells/mL/day. While Porphyridium sp. and Tetraselmis chuii were found to be 0.61 x 10⁴ cells/mL/day and 0.56 x 10⁴ cells/mL/day. The optimum biomass productivity of microalgae Nannochloropsis oculata, Porphyridium sp. and Tetraselmis chuii was found to be day, respectively.

Keywords: Marine microalgae, Urea, Seawater, Biofuel.

1 Introduction

The global energy crisis has become a major focus of public discussions and government policies worldwide [1]–[4]. The need for more energy, combined with the high expense of fossil fuels and the adverse environmental impact of greenhouse gas emissions, has led to a search for creative and sustainable solutions [5] [6]. Researchers have investigated alternative energy sources such as bioenergy, which produces biodiesel and is deemed more economically efficient, socially responsible, and environmentally friendly [7]. Studies on alternative solutions
for bioenergy have included examining biodiesel products from fungi, castor plants, black soldier fly larvae, lignocellulose, and microalgae [7]–[11]. Given their high productivity levels, microalgae are viewed as a promising source of biodiesel [12].

Microalgae have potential as a source of energy for producing biofuels made from biomass [13]. As third-generation biofuels, microalgae offer advantages over other bioenergy products, including fast growth, resilience to diverse environments, and lipid content suitable for biodiesel [14]. Previous studies have investigated various types of microalgae, including Micractinium sp., Tetradesmus obliquus, Chlorella vulgaris, Dunaliella parva, Choricystis minor, and Monoraphidium contortum [15]–[20]. However, many of these studies focused on freshwater microalgae, with limited research on marine microalgae. Notably, marine microalgae such as Nannochloropsis oculata have been found to have higher lipid content than freshwater microalgae like Chlorella vulgaris [21]. The study of marine microalgae's potential for biodiesel is crucial in reducing the pressure on diminishing freshwater resources.

The utilization of microalgae as biodiesel offers environmental benefits, including the reduction of nitrogen in wastewater [22]. Excessive use of urea, one of the most commonly used nitrogen fertilizers in agriculture, can pollute soil and water [23]. Microalgae can absorb nitrogen, including urea, so selecting the right strain is critical in wastewater treatment [24], [25]. Therefore, employing microalgae for biodiesel production can help absorb nitrogen in the environment, supplementing their use as a renewable energy source.

Marine microalgae, such as Nannochloropsis oculata, Tetraselmis chuii, and Porphyridium sp., have shown great promise for biodiesel production due to their ability to produce high yields of biomass [26], [27]. To maximize their biodiesel potential and minimize urea waste, it is crucial to test the cultivation of these microalgae under synthetic urea waste pressure. The objective of this study is to investigate the growth rate and biomass yield of three marine microalgae species, namely Nannochloropsis oculata, Tetraselmis chuii, and Porphyridium sp. using synthetic urea waste water media.

2 Method

2.1 Microalgae Cultivation

The study employed Nannochloropsis oculata (A), Tetraselmis chuii (B), and Porphyridium sp. (C) strains of microalgae, obtained from the microalgae cultivation collection located at the Center for Marine Aquaculture Fisheries in Lampung, Indonesia. Optical microscopy, combined with a hemocytometer and hand counter was used to count each strain of microalgae [28]. The algae strains were diluted into sterile seawater to achieve a cell density of 30 x 10^4 cells/mL for Nannochloropsis oculata, 7 x 10^5 cells/mL for Tetraselmis chuii, and 3 x 10^4 cells/mL for Porphyridium sp.
2.2 Microalgae Cultivation

Algae strains were grown in 2L glass vials filled with 1 L sterile seawater medium. To aid in the adaptation of microalgae to the new environment, 10 mg/L of Triple Super Phosphate (TSP) fertilizer was added. All media and equipment were sterilized by autoclaving at 121°C for 20 minutes prior to use to eliminate any external contaminants. During the experiment, we closely monitored environmental parameters including salinity (27.21±0.99 ppt), temperature (24.31±0.87 °C), pH (6.26±0.43), and dissolved oxygen (7.76±0.41 mg/L).

2.3 Synthetic Urea addition

Synthetic urea (CH₄N₂O) Pro Analis was diluted in 1 L of distilled water using a multilevel dilution system. The urea concentration was divided into six concentrations, including 25 mg/L (T2), 50 mg/L (T3), 75 mg/L (T4), 100 mg/L (T5), 150 mg/L (T6) as the primary parameter and the control variable (0 mg/L, T1). Each treatment was repeated three times and averaged to obtain precise results. The difference in concentration was intended to observe the effect of urea wastewater media on the growth of microalgae in a laboratory setting.

2.4 Microalgae Kinetic Growth

Microalgal growth was assessed daily by counting cells in 1 mL of culture medium using a light microscope with hemocytometer and hand counter. This process was performed for each microalgae species observed, repeated three times, and then averaged. The equation used to calculate the specific growth rate (\(\mu\)) of each species is as follows: [26]:

\[
\mu = \frac{\ln(N) - \ln(N_0)}{t - t_0}
\]  

(1)

In this equation, \(\mu\) (d⁻¹) represents the specific growth rate during the exponential phase. N represents the cell density at time (t), and N₀ represents the initial density during the exponential phase (t₀). The doubling time (td) of cells was calculated to indicate the mean biomass generation time corresponding to the specific growth rate (\(\mu\)) derived from the formula provided below [26]:

\[
td = \frac{\ln 2}{\mu}
\]  

(2)

The dry weight of microalgae was measured at the start of cultivation and at the point of highest density during cultivation (H-exponential). To do this, 25 mL of media was filtered using GF/C filter paper (90 mm diameter) with the assistance of a 27 kPa vacuum pump. To avoid any moisture that could affect the mass of the filter paper, it was preheated in an oven at 105°C for 1 hour before filtration. After filtration, the filter paper was reheated using the same method. Microalgae biomass productivity is determined by calculating the dry weight of microalgae using the following formula [17]:
Biomass Yield = \frac{(\text{DELP} - \text{DEEP})}{\Delta t} \tag{3}

Where, DELP and DEEP represent the dry weight (g/L) at the end of the exponential period and the beginning of exponential growth.

2.5 Statistical Analysis

Data were analyzed using IBM Statistical Package for the Social Sciences (SPSS) descriptive statistics. One-way analysis of variance (ANOVA) at a significance level of \( p \leq 0.05 \) was used to test daily cell density records for normality and heterogeneity. Tukey's posthoc test was used to compare cell density between different urea concentrations. Pearson's correlation test was used for examination of the relationship between variables.

3 Results and Discussion

3.1 Cell density

The study examined the effect of increasing urea concentrations (0 mg/L, 25 mg/L, 50 mg/L, 75 mg/L, 100 mg/L, and 150 mg/L) on the growth of the microalgae *Nannochloropsis oculata*, *Tetraselmis chuii*, and *Porphyridium* sp.. The batch cultivation had different observation times, with *Nannochloropsis oculata* taking 9 days to reach the desired growth and *Tetraselmis chuii* and *Porphyridium* sp. taking 7 days each. Successful cultivation was indicated by the absence of significant growth inhibition during the exponential phase. This phase involves sequential cell division, resulting in an increase in the specific growth rate that continues until it reaches its peak value \[29\]. This confirms the adaptability of microalgae in the early phase to the urea-polluted environment.

The growth density of *Nannochloropsis oculata* cells during the exponential phase cultivated for 9 days was found to vary with each experiment (Figure 1-A). The experimental strains (A1, A2, A3, A4, A5, A6: initial density of 300 x 10^4 cells/mL) experienced the highest growth at different densities and times, respectively at 2990 x 10^4 cells/mL (Day 5), 4350 x 10^4 cells/mL (Day 6), 4475 x 10^4 cells/mL (Day 7), 4531 x 10^4 cells/mL (Day 7), 4475 x 10^4 cells/mL (Day 7), and 4805 x 10^4 cells/mL (Day 7). Treatment A6 (150 mg/L urea) showed the highest cell density growth of 4805 x 10^4 cells/mL on day 7. In contrast to the *Tetraselmis chuii* strain, which only requires a cultivation period of 7 days (Figure 1-B), each treatment (B1, B2, B3, B4, B5, B6: initial density of 30 x 10^4 cells/mL) reached its optimum density at different times: 282 x 10^4 cells/mL (Day 5), 383 x 10^4 cells/mL (Day 5), 493 x 10^4 cells/mL (Day 5), 571 x 10^4 cells/mL (Day 6), 577 x 10^4 cells/mL (Day 6), and 623 x 10^4 cells/mL (Day 6), respectively. Treatment A6 (150 mg/L urea) also showed the highest density value of 623 x 10^4 cells/mL (Day 6). In contrast, the *Porphyridium* sp strain, cultivated for 7 days at an initial cell density of 70 x 10^4 cells/mL, showed a different optimum cell density (Figure 1-C). The optimum cell density for each
treatment was $529 \times 10^4$ cells/mL (C1; day 5), $729 \times 10^4$ cells/mL (C2; day 5), $2335 \times 10^4$ cells/mL (C3; day 6), $2480 \times 10^4$ cells/mL (C4; day 6), $3037 \times 10^4$ cells/mL (C5; day 6), and $3787 \times 10^4$ cells/mL (C6; day 6). The treatment with the highest cell density was C6, which had 150 mg/L urea added on day 6, with a value of $3787 \times 10^4$ cells/mL.

Fig. 1. Microalgae cell density ($10^4$ cells/mL). A) *Nannochloropsis oculata*; B) *Tetraselmis chuii*; C) *Porphyridium sp.*
The study results show that adding urea affects the growth of cell density in each microalgae strain. Nannochloropsis oculata strain (treatment A6) experienced 1.6 times more cell density growth than non-urea treated cells (A1). *Tetraselmis chuii* and *Porphyridium sp* strains both experienced significant increases in cell density growth compared to the control variable. Specifically, *Tetraselmis chuii* saw a growth of 2.21 times the cell density of the control, while *Porphyridium sp* saw a growth of 7.02 times. The statistical tests (one-way ANOVA) for each species revealed no significant difference in the provision of urea concentrations between *Nannochloropsis oculata* and *Tetraselmis chuii* species (p=0.25>0.05 and p=0.796>0.05). Therefore, it can be assumed that urea did not have a major influence on the growth of these two types of microalgae. In *Porphyridium sp.*, the application of urea with varying concentrations significantly affects microalgae cell growth (p=0.01<0.05). This finding is consistent with previous research showing that an enhancement in the growth of microalgae species, such as Coccomyxa acidophila, can be significantly increased by the addition of urea [30].

The addition of urea at 150 mg/L dominated the significant growth of microalgae density. This is because microalgae can utilize urea as a nitrogen source for nutrients [31]. Urea seems to promote better microalgae growth compared to ammonia [32]. However, a lack of available nitrogen can lead to nutrient deficiencies, resulting in a decrease in the growth rate of microalgae [28]. In this study, each control variable (without treatment) has the lowest density in each species, which is the defining condition. Consequently, increased cell density growth can thrive at higher urea concentrations (150 mg/L).

### 3.2 Specific growth rate (μ) and doubling time (dt)

The specific growth rates (μ) of the three algal species examined varied considerably (Figure 2). At a concentration of 150 mg/L, the highest values were recorded for all three species: 0.44 day⁻¹, 0.25 day⁻¹, and 0.76 day⁻¹, respectively, with doubling times (dt) of 1.5 days, 2.72 days, and 0.96 days (Figure 3). Compared to the control variable, the specific growth rate only reached 0.12 day⁻¹, 0.06 day⁻¹, and 0.14 day⁻¹. Compared to *A. carterae* tested in urea wastewater, *Tetraselmis chuii* had a lower μmax value of 0.30 ± 0.08 day⁻¹ [33]. The assay determined that *Tetraselmis chuii* had a maximum growth rate (μmax) of 0.686 μmax/day under varying salinity conditions [34], [35]. This difference is believed to be primarily due to variations in nutrient availability which affects the response of microalgae to nutrient absorption in their environment and subsequently impacts growth rates [36].
The doubling time analysis revealed that the *Tetraselmis chuii* species had the highest value compared to other species and concentrations. Specifically, the control variable and 25 mg/L urea concentration required 11.7 days and 10.9 days, respectively. The Pearson correlation showed a correlation (sig<0.05) of the specific growth rate with doubling time, resulting in a correlation coefficient of -0.745. Based on this, there is a strong inverted linear correlation between both variables.

3.3 Biomass Yield

The microalgae species evaluated (*Nannochloropsis oculata, Tetraselmis chuii,* and *Porphyridium* sp.), achieved their highest biomass yield rate at a urea concentration of 150 mg/L. Specifically, the production rates were 821.33 g/m³/day, 522.94 g/m³/day, and 1244.4 g/m³/day, respectively (Figure 4). A Pearson correlation analysis between the specific growth
rate and biomass production revealed a strong correlation (sig<0.05) with a correlation coefficient of 0.918. Environmental conditions such as light exposure, temperature variations, necessary nutrients, carbon dioxide availability, system pH, and salinity can influence the growth of microalgae, which is crucial for biodiesel fuel production [37]. The addition of urea at all replacement levels can increase protein, while the carbohydrate content in N. oc-ulata decreases. Microalgal species tend to have a decrease in carbohydrate amount as protein quantity increases [38]. Triglyceride accumulation in cells is higher with an increase in alkaline pH. Conversely, acidic pH can facilitate changes in nutrient uptake or create pollutants that are harmful to algal growth [39]. Environmental conditions affect nutrient absorption, including the addition of urea as a nutrient in the microalgae medium.

Fig. 4. Biomass yield (g/m³/day)

The study was limited to biomass, and although lipids were not studied, the state of biomass can be used as a reference in biodiesel production. Previous research has shown that *Nannochloropsis oculata* has a biodiesel conversion percentage of 85.12% [40]. Our findings indicate that *Porphyridium sp* microalgae has higher biomass production than *Nannochloropsis oculata* and *Tetraselmis chuii*, making it a potential biodiesel feedstock. Additionally, due to its high biomass production, *Porphyridium sp* microalgae can also be used as a cosmetic raw material [41]. This makes this species potential as a biodiesel feedstock because it has high biomass production.
4 Conclusion

The research findings show that Nannochloropsis oculata microalgae had a growth rate of 0.44 x 10^4 cells/mL/day, while Porphyridium sp. and Tetraselmis chuii had growth rates of 0.61 x 10^4 cells/mL/day and 0.56 x 10^4 cells/mL/day, respectively. Additionally, Nannochloropsis oculata, Porphyridium sp., and Tetraselmis chuii had optimal biomass productivity rates of 82.3 g/m^3/day, 552.94 g/m^3/day, and 1244.4 g/m^3/day, respectively. The utilization of microalgae shows promise in mitigating the negative effects of urea on marine water pollution.

References


